IDENTIFYING MATERIAL FROM A BREAST DUCT

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of application no. 09/502,404, filed on February 10, 2000, which was a continuation-in-part of application no. 09/313,463, filed on May 17, 1999, which claimed the benefit of provisional application no. 60/117,281, filed on January 26, 1999. This application is also a continuation-in-part of application no. 09/473,510, filed on December 28, 1999. This application also claims the benefit under 37 CFR 1.78 of provisional application 60/166,100 filed on November 17, 1999. The full disclosures of each of the prior applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The field of this invention is methods and systems for detecting breast cancer and breast precancer in humans.

2. Description of the Background Art

For several decades significant members of the medical community dedicated to studying breast cancer have believed and shown that the cytological analysis of cells retrieved from nipple discharge from the breast milk ducts can provide valuable information leading to an identifying patients at risk for breast cancer. Indeed Papanicolaou himself contributed to the genesis of such a possibility of a "Pap" smear for breast cancer by analyzing the cells contained in nipple discharge. See Papanicolaou et al, "Exfoliative Cytology of the Human Mammary Gland and Its Value in the Diagnosis of Cancer and Other Diseases of the Breast" Cancer (1958) March/April 377-409. See also Petrakis, "Physiological, biochemical, and cytological aspects of nipple aspirate fluid", *Breast Cancer Research and Treatment* 1986; 8:7-19; Petrakis, "Studies on the epidemiology and natural history of benign breast disease and breast cancer using nipple aspirate fluid" *Cancer Epidemiology, Biomarkers and Prevention* (Jan/Feb 1993) 2:3-10; Petrakis, "Nipple Aspirate Fluid in epidemiological studies of breast disease", *Epidemiologic Reviews* (1993) 15:188-195. More recently, markers have also been detected in nipple fluid. See Sauter *et al*, "Nipple aspirate fluid: a promising non-invasive method to identify cellular markers of breast cancer risk", *British Journal of Cancer*

76(4):494-501 (1997). The detection of CEA in fluids obtained by a nipple blot is described in Imayama *et al.* (1996) *Cancer* 78: 1229-1234.

Breast cancer is believed to originate in the lining of a single breast milk duct in the breast; and additionally human breasts are believed to contain from 6 to 9 of these ducts. See Sartorius , *JAMA* 224 (6): 823-827 (1973). Sartorius describes use of hair-like single lumen catheters that are inserted into breast ducts using an operating microscope and the ducts were flushed with saline solution as described in Cassels, D March 20th, 1973, *The Medical Post*, article entitled "New tests may speed breast cancer detection". Sartorius *et al*, Contrast ductography for recognition and localization of benign and malignant breast lesions: an improved technique. pp. 281-300. In: Logan WW, ed. *Breast Carcinoma* New York, Wiley, 1977. After the fluid was infused, the catheter was removed because it was too small to collect the fluid, the breast was squeezed and fluid that oozed onto the nipple surface was removed from the surface by a capillary tube. Similarly, Love and Barsky, "Breast-duct endoscopy to study stages of cancerous breast disease", *Lancet* 348(9033):997-999, 1996 describes cannulating breast ducts with a single lumen catheter and infusing a small amount of saline, removing the catheter and squeezing to collect the fluid that returns on the nipple surface. The use of a rigid 1.2 mm ductscope to identify intraductal papillomas in women with nipple discharge is described in Makita *et al* (1991) *Breast Cancer Res Treat* 18: 179-188. It would be advantageous to develop methods and devices to collect the ductal fluid from within the duct.

Galactography, or contrast ductography has for years located breast ducts based on spontaneous nipple discharge, infused the ducts (using cannulas for this purpose) with contrast dye solutions, and taken x-ray pictures to determine the source of the discharge within the duct. See generally, The Breast: Comprehensive Management of Benign and Malignant Breast Diseases, Bland and Copeland eds. W.B. Saunders Co. Philadelphia PA 1991 pages 61-67.

Nuclear matrix proteins are implicated in bladder, colon, prostate, breast and other cancers, and have been put forth by Matritech, Inc (Newton, MA 02460) as part of a kit for testing for bladder cancer using body fluid. For testing for breast cancer, a blood test has been developed using antibodies to nuclear matrix proteins (see website for Matritech, Inc. http://www.matritech.com) The blood and body fluid tests are promoted as being capable of early detection of the cancers they seek to identify. In addition, profiles and differential patterns of expression of nuclear matrix proteins have been

detected for several different cancers (Fey and Penman 1986, Stuurman 1990, and Getzenberg 1990).

Matritech, Inc. has patented various aspects of proteins and nucleic acids of nuclear matrix proteins as well as kits for testing for their presence in order to identify cancer in U.S. Patent Nos. 5,965,376, 5,914,238, 5,882,876, 5,858,683, 5,840,503, 5,830,677, 5,783,403, 5,780,596, 5,698,439, 5686,562, and 5,547,928. Specifically, Matritech has patented claims in U.S. Patent No. 5,914,238 to a method for diagnosing breast cancer in a patient comprising detecting the presence of a breast cancer-associated protein in a tissue or body fluid obtained from the patient. The breast cancer-associated protein has a molecular weight of about 32,500 or 33,000 Daltons and an isoelectric point of about 6.82, and has a continuous amino acid sequence from several amino acid sequences disclosed in the application. The nuclear matrix protein is detected by polyclonal or monoclonal antibodies or by PCR amplification of an expression product of the target gene. The patient sample in the examples is breast tissue samples, although the possibility of testing blood or body fluid is claimed and mentioned in the specification. Testing breast duct fluid is not described.

SUMMARY OF THE INVENTION

An object of the invention is to identify a patient having breast cancer or breast precancer. In accordance with this object, is provided a method comprising providing a ductal fluid sample from one duct of a breast of a patient, the fluid not mixed with ductal fluid from any other duct of the breast, and examining the ductal fluid sample to determine the presence of a marker comprising a protein, a polypeptide, a peptide, a nucleic acid, a polynucleotide, an mRNA, a small organic molecule, a lipid, a fat, a glycoprotein, a glycopeptide, a carbohydrate, an oligosaccharide, a chromosomal abnormality, a whole cell having a marker molecule, a particle, a secreted molecule, an intracellular molecule, and a complex of a plurality of molecules. In accordance with this object is also provided methods for determining markers which can identify a patient having breast cancer or precancer by examining the ductal fluid sample to determine the presence of a marker comprising RNA, DNA, protein, polypeptide, or peptide form of the marker. The invention also includes a method of identifying a patient having breast cancer or breast precancer, said method comprising providing a ductal fluid sample from one duct of a breast of a patient, said fluid not mixed with ductal fluid from any other duct of the breast, examining the ductal fluid sample to determine the presence of a

marker comprising a protein, a polypeptide, a peptide, a nucleic acid, a polynucleotide, an mRNA, a small organic molecule, a lipid, a fat, a glycoprotein, a glycopeptide, a carbohydrate, an oligosaccharide, a chromosomal abnormality, a whole cell having a marker molecule, a particle, a secreted molecule, an intracellular molecule, and a complex of a plurality of molecules; wherein the marker is capable of differentiating between any two of cytological categories consisting of normal, abnormal, hyperplasia, atypia, ductal carcinoma, ductal carcinoma in situ (DCIS), ductal carcinoma in situ - low grade (DCIS-LG), ductal carcinoma in situ - high grade (DCIS-HG), invasive carcinoma, atypical mild changes, atypical marked changes, atypical ductal hyperplasia (ADH), insufficient cellular material for diagnosis, and sufficient cellular material for diagnosis.

Any of the methods can comprise further analyzing the ductal fluid for abnormal cytology. Any of the methods can comprise that the ductal fluid is retrieved by placing a ductal access tool in the duct and infusing fluid into the duct through the tool and retrieving from the accessed duct through the tool a portion of the infused fluid mixed with ductal fluid. The method can be repeated for more than one duct on a breast; for example, the method can be practiced comprising a plurality of ducts on a breast.

The invention provides additionally, a method for identifying a patient having breast cancer or breast precancer, by providing a ductal fluid sample from at least one duct of a breast of the patient; and examining the ductal fluid sample to determine the presence of a marker comprising an expression product of a gene encoding a nuclear matrix protein. The expression product can comprise a nucleic acid or a polypeptide. The expression product can comprise RNA, or a protein or a part of a protein. The nuclear matrix protein can be lamin A, lamin B, lamin C, a peripheral matrix protein, nuclear mitotic spindle apparatus protein (NuMA), topoisomerase II, or an internal nuclear matrix protein. The expression product can be a polypeptide and examining can comprise contacting the polypeptide marker with an antibody that specifically binds a portion of the polypeptide. The expression product can be a nucleic acid and examining can comprise detecting the presence of the nucleic acid. Detecting the presence of the nucleic acid can comprise amplifying the nucleic acid. Providing the ductal fluid sample can comprise obtaining the sample from the breast. Providing the ductal fluid sample can comprise receiving a sample that had been previously obtained. The fluid can be obtained by nipple aspiration of the milk ducts. The fluid sample can be obtained by washing the ductal lumen and retrieving fluid and cells from the lumen. The fluid collected can be from a single duct. The fluid can be collected from a plurality of ducts.

The invention also provides systems for diagnosing breast cancer or precancer comprising a tool to retrieve ductal fluid from a breast duct and instructions for use to determine the presence of a marker.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

The following preferred embodiments and examples are offered by way of illustration and not by way of limitation.

The method of the invention provides a method of screening women for breast cancer or precancer comprising providing a ductal fluid sample from at least one duct of a breast of the patient; and detecting an increased level of a marker wherein an increased level of one or more markers indicates an increased risk for breast cancer or precancer.

The method is practiced by providing a ductal fluid sample from at least one duct of a breast of the patient. Providing the ductal fluid sample can comprise obtaining the sample from the breast. Providing the ductal fluid sample can also comprise receiving a sample that had been previously obtained. For example, a laboratory can receive a ductal fluid sample from a patient or a practitioner, and the laboratory can be directed to make an analysis of the sample. Where the fluid is obtained from the breast, the fluid sample can be obtained e.g. by nipple aspiration of the milk ducts or by ductal lavage of at least one breast milk duct. When fluid is collected by nipple aspiration, or by ductal lavage, the fluid can be collected from a single duct. For example the duct and the collection tube can be marked so that the analysis of the fluid is traceable to one duct.

By the procedure of ductal lavage, ductal epithelial cells that line the walls of the ductal lumen are washed out of the duct. Lavage or wash fluid is infused into the duct, and the lavage fluid mixed with ductal fluid is collected. Lavage is described in copending and co-owned applications including 09/067,661, 09/301,058, PCT US99/09141, 60/122,076, 09/313,463, 60/143,359, and U.S. Application No. 09/473,510, all incorporated by reference in their entirety. In some cases suction can be applied to the tool accessing the ductal lumen in order to retrieve a maximum amount of cells and/or fluid. Lavage or wash fluid can be infused into the duct, and collected. Suction can be applied to the tool accessing the ductal lumen in order to retrieve a maximum amount of cells and/or fluid.

Access of a breast duct can be facilitated as described in e.g. Love & Barsky, (1996) *Lancet* 348: 997-999, Makita *et al* (1991) *Breast Cancer Res Treat* 18: 179-188, or Okazaki *et al* (1991) *Jpn J. Clin. Oncol.* 21:188-193. Alternatively, ductal fluid can be retrieved by a medical tool, e.g. a catheter or a cannula placed into the duct to infuse wash fluid to retrieve a mixture of wash and ductal fluids. The fluid from the breast duct can contain ductal epithelial cells, including cells of a stage considered to be precancerous or cancerous.

Nipple aspiration of breast ductal fluid is achieved by using vacuum pressure. Nipple aspiration techniques are also described and claimed in co-pending and co-owned patent application U.S. Patent Application No. 09/438,219, herein incorporated by reference in their entirety. Nipple aspirate fluid can be retrieved as described in e.g. Goodson WH & King EB, *Chapter 4: Discharges and Secretions of the Nipple* , The Breast: Comprehensive Management of Benign and Malignant Diseases (1998) 2nd Ed. vol 2, Bland & Kirby eds. W.B. Saunders Co, Philadelphia, PA pp. 51-74; Wrensch et al., (1992) American Journal of Epidemiology. 135(2):130-41; and Sauter et al (1997) British Journal of Cancer. 76(4):494-501. Ductal lavage is described in copending patent application USSN 09/067,661 filed April 28th, 1998. Cells of the lesion can be retrieved by collecting the ductal fluid that contains some of these cells, e.g. by aspirating the nipple to obtain nipple aspirate fluid, e.g. as described in Petrakis (1993) *Cancer Epidem. Biomarker Prev.* 2:3-10, Petrakis (1986) *Breast Cancer Res. Treat* 8: 7-19, Wrensch *et al* (1992) *Am. J. Epidem.* 135:130-141, Wrensch *et al* (1990) *Breast Cancer Res Treat* 15: 39-21, and Wrensch *et al* (1989) *Cancer Res.* 49: 2168-2174. Also fluid secretions from the nipple can be collected as they spontaneously appear on the nipple surface. In order to collect the fluid not mixed with ductal fluid from other ducts, a practitioner carefully watches for the signs of fluid and retrieves the fluid from the nipple surface near the orifice before it has a chance to mix with fluid from any other orifice.

The ductal fluid may be analyzed *in situ*, i.e. inside the breast and inside the breast duct, e.g. where a particular marker can be introduced into the duct and can be identified from within the breast. *In situ* testing within the duct is also considered a non-invasive means of examining the ductal epithelial cells. Ductal epithelial cells that are examined by the method of the invention can be examined *in situ* (i.e. in the duct; e.g. where a marker can bind the cells or a component of the cells in the duct and be identified from within the breast by a tag attached to the marker), or after the ductal epithelial cells have been removed from the breast of the patient by non-invasive means, e.g. as just

described. Methods of *in situ* analysis can include use of such molecular biology tools, methods, and materials as described in e.g. U.S. Patent Nos. 5,169,774, 5,720,937, 5,677,171, 5,720,954, 5,725,856, 5,770,195, and 5,772,997. Markers to breast cancer and breast precancer described elsewhere and herein may also be used for an *in situ* analysis of the breast duct.

The ductal fluid is examined to detect the presence of precancerous or cancerous ductal epithelial cells. The fluid sample (comprising ductal epithelial cells) can be analyzed by any effective means for identifying breast precancer or cancer, including e.g. cytological analysis of the cells retrieved or identified. Examination of the ductal epithelial cells can be accomplished by examining useful indicators such as, e.g. the morphology of the cells or the cellular contents. The cellular contents can include, e.g. protein, nucleic acid, particles, complexes or other biochemical or molecular markers in the cells. Cell morphology can serve to establish whether the ductal epithelial cells are normal (i.e. not precancerous or cancerous or having another noncancerous abnormality), precancerous (i.e. comprising hyperplasia, atypical ductal hyperplasia (ADH) or low grade ductal carcinoma *in situ* (LG-DCIS)) or cancerous (i.e. comprising high grade ductal carcinoma *in situ* (HG-DCIS), or invasive carcinoma).

Analysis of cell contents may serve to establish similar staging as established by morphology, capturing generally a progression of a precancerous or cancerous condition in the cells. Thus the ductal epithelial cells may be analyzed for other markers, e.g. protein markers, nucleic acid markers, particles, complexes, or biochemical or molecular markers in the cells or on the cell surfaces or secreted by the cell or for any marker providing evidence of neoplasia. The ductal epithelial cell can be derived from any part of the breast milk duct, including, e.g. the ductal lumen and/or the terminal ductal lobular unit (TDLU). Cells derived from the TDLU may also have similar stages as found in other luminal ductal epithelial cells not from the TDLU including, e.g. hyperplasia, atypia, in situ carcinoma, and invasive carcinoma.

Once the wash fluid had been infused in the duct and the wash fluid and ductal fluid is collected from a breast duct, the cellular material can be separated and can be examined. The cellular material can include, e.g. substances selected from the group consisting of whole cells, cellular debris, proteins, nucleic acids, polypeptides, glycoproteins, lipids, fats, glycoproteins, small organic molecules, metabolites, and macromolecules. Cytology, or any other suitable method for analyzing the condition of the cells can examine whole cells. Other markers present in the cellular material, ductal

fluid generally, or other material obtained from the breast duct can be analyzed as is appropriate for the marker being sought, including e.g. binding assays, immunohistochemistry, or using other analytical technology for distinguishing and identifying biological molecules obtained from biological material.

Identifying a patient having breast cancer or breast precancer can be accomplished by removing breast duct fluid from the patient and analyzing the fluid comprising ductal contents for markers that may indicate a cancerous or precancerous condition in the breast. Providing a ductal fluid sample from one duct of a breast of a patient includes that the fluid is not mixed with ductal fluid from any other duct of the breast. The method is practiced by providing a ductal fluid sample from at least one duct of a breast of the patient. Providing the ductal fluid sample can comprise obtaining the sample from the breast. Providing the ductal fluid sample can also comprise receiving a sample that had been previously obtained. For example, a laboratory can receive a ductal fluid sample from a patient or a practitioner, and the laboratory can be directed to make an analysis of the sample. Where the fluid is obtained from the breast, the fluid sample can be obtained e.g. by nipple aspiration of the milk ducts or by ductal lavage of at least one breast milk duct. When fluid is collected by nipple aspiration, or by ductal lavage, the fluid can be collected from a single duct. For example the duct and the collection tube can be marked so that the analysis of the fluid is traceable to one duct.

The ductal fluid can be retrieved by placing a ductal access tool in the duct and infusing fluid into the duct through the tool and retrieving from the accessed duct through the tool a portion of the infused fluid mixed with ductal fluid. The process may be repeated for more than one duct on a breast, and/or the process can be repeated for a plurality of ducts on a breast. Either sequential or simultaneous access of the duct on a breast can be used.

The next step in the method after the fluid is collected is examining the ductal fluid sample to determine the presence of a marker comprising a protein, a polypeptide, a peptide, a nucleic acid, a polynucleotide, an mRNA, a small organic molecule, a lipid, a fat, a glycoprotein, a glycopeptide, a carbohydrate, an oligosaccharide, a chromosomal abnormality, a whole cell having a marker molecule, a particle, a secreted molecule, an intracellular molecule, and a complex of a plurality of molecules.

Examining the ductal fluid sample can comprise determining the presence of a marker comprising RNA, DNA, protein, polypeptide, or peptide form of a marker

selected from the group consisting of a receptor, a ligand, a protein factor, an antigen, an antibody, an enzyme, a soluble protein, a cytosolic protein, a cytoplasmic protein, a tumor suppressor, a cell surface antigen, a phospholipid, a lipoprotein, a hormone responsive protein, a differentiation associated antigen, a proliferation associated antigen, a metastasis associated antigen, an integral membrane protein, a protein that participates in an apostasis pathway, a protein that participates in a transcriptional activation pathway, a cell adhesion molecule, an extracellular matrix protein, a proteolipid, a cytokine, a basement membrane protein, a mucin-type glycoprotein, a histone, a ribonucleoprotein, a sialic acid, a bone matrix protein, a carbohydrate antigen, a nuclear protein, a nuclear phosphoprotein, a proto-oncogene, an oncogene, an apolipoprotein, a serine protease, a tumor rejection antigen, a surfactant protein, a cell death protein, a zinc endoprotease, and a trefoil gene.

Examining the ductal fluid sample can comprise determining the presence of a marker comprising RNA, DNA, protein, polypeptide, or peptide form of a marker selected from the group consisting of a chemokine, a lectin, an integrin, a selectin, a keratin, an interleukin, a taxin, a ferritin, a lipocalin, a laminin, a cyclin, a relaxin, a nuclein, a caspase, a melanoma-associated antigen, a macrophage inflammatory protein, a gap junction protein, a calcium binding protein, an actin binding protein, a phospholipid binding protein, a heat shock protein, a cell cycle protein, an activator of tyrosine and tryptophan hydroxylase, a member of the tumor necrosis factor family of proteins, a member of the transforming growth factor alpha family of proteins, a member of the transforming growth factor beta family of proteins, a member of the Bcl2 family of proteins, a Bcl2-interacting protein, a Bcl2-associated protein, a member of the vasopressin/oxytocin family of proteins, and a member of the CCAAT/enhancer binding protein family of proteins.

Examining the ductal fluid sample can comprise determining the presence of a marker wherein the marker is an enzyme and the enzyme comprises an RNA, DNA, protein, polypeptide, or peptide form of an enzyme selected from the group consisting of a phosphorylase, a phosphatase, a decarboxylase, an isoenzyme, a kinase, a protease, a nuclease, a peptidase, a protease, a DNase, an RNase, an aminopeptidase, a topoisomerase, a phosphodiesterase, an aromatase, a cyclooxygenase, a hydroxylase, a dehydrogenase, a metalloproteinase, a telomerase, a reductase, a synthase, an elastase, a tyrosinase, a transferase, and a cyclase.

Examining the ductal fluid sample can comprise determining the presence of a marker wherein the marker is a receptor and the receptor comprises an RNA, DNA, protein, polypeptide, or peptide form of a receptor selected from the group consisting of a steroid hormone receptor, a growth factor receptor, a kinase receptor, a G-protein linked receptor, a TNF family receptor, a tyrosine kinase receptor, a vasopressin receptor, an oxytocin receptor, and a serine protease receptor.

Examining the ductal fluid sample can comprise determining the presence of a marker wherein the marker is a protein factor and the factor comprises an RNA, DNA, protein, polypeptide, or peptide form of a factor selected from the group consisting of a growth factor, a proteolytic factor, a stromal cell factor, an epithelial cell factor, an angiogenesis factor, an epithelial cell factor, an angiogenic factor, and a colony stimulating factor.

Examining the ductal fluid sample can comprise determining the presence of a marker wherein the marker is an inhibitor and the inhibitor comprises an RNA, DNA, protein, polypeptide, or peptide form of an inhibitor selected from the group consisting of an inhibitor of a cyclin, an inhibitor of a cyclin complex, a serpin, an inhibitor of proteolytic degredation, a tissue inhibitor of a metalloprotease, and an angiogenesis inhibitor.

Examining the ductal fluid can comprise identifying a level or quality of at least one marker comprising an expression product of a gene encoding a nuclear matrix protein.

A level of the marker can be a presence relative to a normal control or an absence relative to a normal control of a given marker. The normal control can be determined relative to the particular patient, or relative to a patient population.

In addition, the quality of the marker can be assessed. A quality of a marker can be such changes as DNA mutation, or a quantity of mutations, a deterioration of chromosomal quality or quantity, degradation of a protein, or a change in quantity of a nucleic acid or chromosome. A quality can be an erosion of a molecule, particle, molecule or organelle with respect to a normal quality.

Once the wash fluid had been infused in the duct and the wash fluid and ductal fluid is collected from a breast duct, the cellular material can be separated and can be examined. The cellular material can include, e.g. substances selected from the group consisting of whole cells, cellular debris, proteins, nucleic acids, polypeptides, glycoproteins, lipids, fats, glycoproteins, small organic molecules, metabolites, and

macromolecules. Examining the ductal fluid sample to determine the presence of a marker comprising a protein, a polypeptide, a peptide, a nucleic acid, a polynucleotide, an mRNA, a small organic molecule, a lipid, a fat, a glycoprotein, a glycopeptide, a carbohydrate, an oligosaccharide, a chromosomal abnormality, a whole cell having a marker molecule, a particle, a secreted molecule, an intracellular molecule, and a complex of a plurality of molecules. Detection and analysis of these classifications of markers can be accomplished as described below, using standard assays for determining the presence of markers or marker classifications listed, for example as described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed. (Cold Spring Harbor Press, Cold Spring Harbor, NY 1989).

Cytology, or any other suitable method for analyzing the condition of the cells can examine whole cells. Other markers present in the cellular material, ductal fluid generally, or other material obtained from the breast duct can be analyzed as is appropriate for the marker being sought, including e.g. binding assays, immunohistochemistry, or using other analytical technology for distinguishing and identifying biological molecules obtained from biological material.

Intracellular components, either secreted or non-secreted and which are found in the ductal fluid may be tested as well. For example, ring-shaped particles which comprise protein, DNA, and RNA can be identified using an assay and/or a binding immogen as described in U.S. Patent Nos. 5,635,605, and 5,459,035, and EP 465,715, including (as described) an affinity chromatography medium specific for proteins with particular characteristics (in the case of the ring-shaped particle a dinucleotide fold) and a standard immunoassay proceeding as described. In addition, complexes of proteins or other molecules may be identified. For example, antibodies may be used to bind complexes, e.g. extracellular matrix complexes in order to identify such a complex that is considered a breast cancer marker. The process of identification of such complexes is described in WO 96/12192.

Exemplary markers are described in Masood S., (Prediction of recurrence for advanced breast cancer. Traditional and contemporary pathologic and molecular markers) *Surgical Oncology Clinics of North America.* 4(4):601-32, 1995; Lopez-Guerrero *et al* (1999) *J Hematother* 8(1):53-61; Marjumdar and Diamandis (1999) *Br J Cancer* 79(9-10):1594-602; Balleine *et al* (1999) *Br J Cancer* 79 (9-10):1564-71; Houston *et al* (1999) *Br J Cancer* 79(7-8):1220-6; Nikolic-Vukosavljevic *et al* (1998) *Tumori* 84(6):691-4; Maguire *et al* (1998) *Int J Biol Markers* 13(3):139-44; Stearns *et al* (1998) *Breast Cancer*

Res Treat 52(1-3):239-59; Eiriksdottir *et al* (1998) *Eur J Cancer* 34(13):2076-81, and U.S. Patent No. 5,169,774. Many known breast cancer markers are discussed and described in readily available medical textbooks on breast cancer. In addition, several markers can be identified and analyzed in the same sample, e.g. Fabian *et al* 1993 *J. Cellular Biochemistry* 17G:153-16 and Fabian *et al* 1994 *Breast Cancer Res Treat* 30(3):263-74 looking at estrogen receptor (ER), epidermal growth factor receptor (EGFR), mutant p53, HER-2 neu by immunohistochemistry and aneuploidy by image analysis in fine needle aspirates. Methods described therein can be practiced by analogy to analysis of ductal fluid contents, particularly ductal epithelial cells retrieved by nipple aspiration and/or by ductal lavage techniques.

Chromosomal abnormalities in ductal epithelial cells can also provide information and act as a marker to identify cancer or precancer as described in Mark *et al* (1999) *Cancer Genet Cytogenet* 108:26-31; Lundlin and Mertens (1998) *Breast Cancer Res Treat* 51:1-15; Newsham (1998) *Am J Pathol* 153:5-9; Larson *et al* (1998) *Am J Pathol* 152:1591-8; Adelaide *et al* (1998) *Genes Chromosomes Cancer* 22:186-99; Fejzo *et al* (1998) *Gene Chromosome Cancer* 22:105-113; Dietrich *et al* (1998) *Hum Pathol* 12: 1379-82; Cavalli *et al* (1997) *Hereditas* 126:261-8; Adeyinka *et al* (1997) *Cancer Genet Cytogenet* 97:119-21; Afify and Mark (1997) *Cancer Genet Cytogenet* 97:101-5; Brenner and Aldaz (1997) *Prog Clin Biol Res* 396: 63-82; Mark *et al* (1997) *Ann Clin Lab Sci* 27:47-56; and Fabian *et al* 1993 *J. Cellular Biochemistry* 17G:153-16.

Other breast cancer markers can be detected as described in Springer, G.F. *et al*, Dao et al, Eds, *Tumor Markers and Their Significance in the Management of Breast Cancer*, pp.47-70, New York; A.R. Liss, 1986. In addition to some markers discussed and/or articles or books cited on breast cancer and breast precancer markers, including markers listed in Porter-Jordan and Lippman, "Overview of the biological markers of breast cancer", Hematology/Oncology Clinics of North America vol. 8 (1):73-100, 1994), the following cancer markers are listed here as exemplary and may be used as well as other markers to analyze the condition of a breast duct, including analysis of the ductal contents (including fluid and cells). Standard assay procedures for identifying the markers can be used, including antibodies or other binding partners, labels, stains, pattern analysis (for cells and cell components), and in general any other chemical or visual identification techniques.

Exemplary markers that are presently being studied by researchers directing their research to breast cancer include, for example, carcinoma embryonic

antigen (CEA), prostate specific antigen (PSA) Erb B2 antigen, gross cystic disease fluid protein -15 (GCDFP-15), and lactose dehydrogenase (LDH). For CEA see Imayama *et al, Cancer* 1996, 78(6):1229-34; Inaji *et al, Cancer* 1987,60(12):3008-13; Mori *Int Conger Seer* 1989, 807:211-8; Inaji, *et al, An To Kagaku Ryoho* 1991, 18(2):313-7; Yayoi, *et al Gan To Kagaku Ryoho* 1994, 21 Suppl 2:133-9; Mori, et al *Jpn J Clin Oncol* 1989,19(4):373-9; Foretova, *et al Proc Annu Meet Am Soc Clin Oncol* 1995,14:A101; and Nishiguchi, *et al Rinsho Byori* 1992,40(1):67-72. For PSA see Foretova and Garber *Lancet* 1996,347(9015):1631; Sauter et al, *Cancer Epidemiology, Biomarkers & Prevention.* 5(12):967-70, 1996; Sauter and Daly (1996) *Proc Annu Meet Am Assoc Cancer Res* 37:A1458; and Foretova and Garber (1996) *Proc Annu Meet Am Assoc Cancer Res* 37:A1446. For Erb B2 see Motomura (1995) *Breast Cancer Res and Treat* 33:89-92; and Inaji et al (1993) *Tumour Biol* 14: 271-8. For GCDFP-15 see Petrakis et al (1994) *Proc Annu Meet Am Assoc Cancer Res* 35:A1698. For LDH see Mannello et al (1995) *Cancer* 76:152-4; and Kawamoto (1994) *Cancer* 73:1836-41.

Generally markers can be, for example, a protein, a polypeptide, a peptide, a nucleic acid, a polynucleotide, an mRNA, a small organic molecule, a lipid, a fat, a glycoprotein, a glycopeptide, a carbohydrate, an oligosaccharide, a chromosomal abnormality, a whole cell having a marker molecule, a particle, a secreted molecule, an intracellular molecule, and a complex of a plurality of molecules. These markers can be detected by detecting an RNA, DNA, protein, polypeptide, or peptide form of a marker selected from the group consisting of, for example, a chemokine, a lectin, an integrin, a selectin, a keratin, an interleukin, a taxin, a ferritin, a lipocalin, a laminin, a cyclin, a relaxin, a nuclein, a caspase, a melanoma-associated antigen, a macrophage inflammatory protein, a gap junction protein, a calcium binding protein, an actin binding protein, a phospholipid binding protein, a heat shock protein, a cell cycle protein, an activator of tyrosine and tryptophan hydroxylase, a member of the tumor necrosis factor family of proteins, a member of the transforming growth factor alpha family of proteins, a member of the transforming growth factor beta family of proteins, a member of the Bcl2 family of proteins, a Bcl2-interacting protein, a Bcl2-associated protein, a member of the vasopressin/oxytocin family of proteins, and a member of the CCAAT/enhancer binding protein family of proteins; or selected from the group consisting of a chemokine, a lectin, an integrin, a selectin, a keratin, an interleukin, a taxin, a ferritin, a lipocalin, a laminin, a cyclin, a relaxin, a nuclein, a caspase, a melanoma-associated antigen, a macrophage inflammatory protein, a gap junction protein, a calcium binding protein, an actin binding

protein, a phospholipid binding protein, a heat shock protein, a cell cycle protein, an activator of tyrosine and tryptophan hydroxylase, a member of the tumor necrosis factor family of proteins, a member of the transforming growth factor alpha family of proteins, a member of the transforming growth factor beta family of proteins, a member of the Bcl2 family of proteins, a Bcl2-interacting protein, a Bcl2-associated protein, a member of the vasopressin/oxytocin family of proteins, and a member of the CCAAT/enhancer binding protein family of proteins. An example of a peptide marker is fibrinogen degredation peptide, which can be assayed as described in WO 98/55872. Another marker that may be detected, e.g., by ELISA tests on the ductal fluid is called DR70™. DR70™ may be sought from ductal fluid essentially as described in Wu et al., *J. Immunoassay*, 1998 vol. 19; no. 1:63-72.

The marker may also or alternatively be an enzyme and the enzyme can comprise an RNA, DNA, protein, polypeptide, or peptide form of an enzyme such as for example, a phosphorylase, a phosphatase, a decarboxylase, an isoenzyme, a kinase, a protease, a nuclease, a peptidase, a protease, a DNase, an RNase, an aminopeptidase, a topoisomerase, a phosphodiesterase, an aromatase, a cyclooxygenase, a hydroxylase, a dehydrogenase, a metalloproteinase, a telomerase, a reductase, a synthase, an elastase, a tyrosinase, a transferase, or a cyclase.

The marker may also or alternatively be a receptor and the receptor can comprise an RNA, DNA, protein, polypeptide, or peptide form of a receptor such as for example a steroid hormone receptor, a growth factor receptor, a kinase receptor, a G-protein linked receptor, a TNF family receptor, a tyrosine kinase receptor, a vasopressin receptor, an oxytocin receptor, and a serine protease receptor.

The marker may also or alternatively be a protein factor and the protein factor can comprise an RNA, DNA, protein, polypeptide, or peptide form of a protein factor such as for example a growth factor, a proteolytic factor, a stromal cell factor, an epithelial cell factor, an angiogenesis factor, an epithelial cell factor, an angiogenic factor, or a colony stimulating factor.

The marker may also or alternatively be an inhibitor and the inhibitor can comprise an RNA, DNA, protein, polypeptide, or peptide form of an inhibitor such as for example an inhibitor of a cyclin, an inhibitor of a cyclin complex, a serpin, an inhibitor of proteolytic degredation, a tissue inhibitor of a metalloprotease, and an angiogenesis inhibitor.

The different categories of markers are tested differently depending on the category and possibly also on the location of the marker in the cell (for example, a cell surface protein might be detected differently than a cytoplasmic or nuclear protein). Typically, assays comprising one or more of binding, coloration, precipitation, affinity column selection, in-situ binding, solution phase binding, nucleic acid probe labeling, protein probe labeling, polypeptide probe labeling, peptide probe labeling, and/or a combination or variation of these processes can be used. Standard procedure for conducting such assays generally (e.g. ELISA, RNA or DNA probe hybridization, and other binding or other detection assays) are described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed. (Cold Spring Harbor Press, Cold Spring Harbor, NY 1989).

More specifically, examples of detection of particular markers or classes of markers are described in Table I below that lists exemplary markers and cites references in which those markers are detected. For markers not specifically listed, broad categories such as e.g. proteins, lipids, RNA transcripts, glycoproteins, and other categories can be detected for other specific markers in the same fashion as specific markers in a same or similar category. Some of these specific markers belonging to broader categories are listed in Table I.

In addition to some markers discussed and/or articles or books cited on breast cancer and breast precancer markers, the following cancer markers are listed here as exemplary and may be used as well as other markers to analyze the condition of a breast duct. Standard assay procedures for identifying the markers can be used, including antibodies or other binding partners, labels, stains, pattern analysis (for ceils and cell components), and in general any other chemical or visual identification techniques.

The following are exemplary potential markers for such identification and analysis: cathepsins (including cathepsin D); maspin, fas, fas ligand, tissue inhibitor of matrix metalloproteinas-1 (TIMP-1); chemokines (both C-C and C-X-C type chemokines); collagenases, metalloproteinases, TIMP's, cathepsins, disrupted basement membrane epitopes, stromolysin-3; cytokeratins (e.g. keratin 14, B1, KA1, KA4 and 312C8-1); estrogen and progesterone receptors (or any androgen or other steroid receptor); growth factor receptors for members of the fibroblast growth family (FGF) including FGF1-18, vascular endothelial growth factor (VEGF), insulin-like growth factor -1 (IGF-I), IGF-II, platelet-derived growth factor (PDGF), keratinocyte growth factor (KGF), and epithelial growth factor (EGF); placental growth factor (PLGF),

hepatocyte growth factor (HGF), tumor necrosis factor (TNF), transforming growth factor (TGF) both alpha and beta forms, and angiopoietin, for example; heat shock proteins (HSP) (e.g. HSP27) 27 (HSP27); ErB type 1 tyrosine kinase receptors (e.g. Her2 (an EGF receptor) or any ligand or receptor of the ErbB family of ligands and receptors); integrins, selectins, cadherins, for example (i.e. alpha and beta 3 integrin); keratin-14; known cancer antigens including, for example Ki-67, Ki-S1, p53, nm23, bcl-2, p21 ras, cyclins, and pS2; thrombin receptor activating peptide; urokinase, urokinase-type plasminogen activator (UPA), plasmin antiplasmin; UPA receptor (UPAR), fibrinogen, plasmin activator inhibitor-1 and 2 (PAI-1 and 2); telomerase; antibodies to tumor associated antigen-72 (TAG-72) (e.g. B72.3, B6.2, and TKH2); carcinoembryonic antigen (CEA) (see e.g. EP 319,686); prostate specific antigen (PSA); gross cystic disease fluid protein - 15 (GCDFP-15); lactose dehydrogenase (LDH); chromosomal abnormalities (e.g. aneuploidy or other abnormalities); S1 protein; alkaline phosphatase; myosin; sialyl Tn (STn) glycopeptide (e.g. TAG-72); Tn glycopeptide; and nuclear matrix proteins (as described in provisional patent application filed 11-17-99 docket no. PDH 99-029, herein incorporated by reference in its entirety).

Other breast cancer markers include, e.g. alanine aminopeptidase, alpha 6 integrin, alpha-lactalbumin, AN43 antigen (BB1), annexin 1, anti-Her2, anti-p53, Bad, BAG-1, Bak, Bax, BCA225, Bcl-2, Bcl-x, beta 1-6 branched oligosaccharides, beta-2 microglobulin (BMG), Bfl-1, bone sialoprotein (BSP), C/EBP beta-LIP, Ca 1 antigen, CA27.29, CA M26, CA M29, CA125, CA15.3, CA195, CA19-9, CA50, CA549, Cadherin-11, calcitonin receptor (CTR), cathepsin B, cathepsin L, CD 105, CD24, CD34 (pan-endothelial marker), CD44, CEA, c-met, c-myc, Cox-1, Cox-2, CPP32, cyclic nucleotide phosphodiesterase, cyclin E, DNA topoisomerase II-alpha, DNA topoisomerase II-beta, EGF, EGFR, E-selectin, fast homoarginine-sensitive alkaline phosphatase (FHAP), fatty acid synthase, ferritin, GCDFP-15/BRST-2, Her-2 (extracellular), h-mts1 (S100A4), hsc73, hsp70, hsp90alpha, hsp90beta, ID1, ID3, interleukin-1 beta, keratin 8, keratin 18, keratin 19, laminin, laminin receptor (MluC5), leucine aminopeptidase (LAP), lipid-bound sialic acid (LSA), MAGE-1, MAGE-2, MAGE-3, Man6-P glycoproteins, MCA, Mcl-1, metallothionein (MT), MKP-1, MMP-2, MMP-9, MSA, MUC-2, MUC-3, MUC-6, Nm23, ornithine decarboxylase (ODC), osteopontin (OPN), P114 (MAR binding protein), P120 (a nucleolar proliferation antigen), p125FAK, p330d/CENP-F (a marker for cell proliferation), PAI-2, Pepsinogen C, placental alkaline phosphatase (PLAP), platelet factor 4 (angiogenic marker), protein

kinase C (PKC), PSA, pyrimidine nucleoside phosphorylase, ras p21, reduced glutathione (GSH), retinoid X receptor alpha, ribosomal S2 protein, sialyltransferase, Stromelysin-1 (MMP-3), surfactant proteins A, surfactant proteins B, TAG-12, TFF-1, TFF-3 (also called ITF, hP1.B and is another trefoil protein besides pS2), thrombin, thrombomodulin, thymidine phosphorylase (TP), thymosin beta 15, tissue cytosol ferritins, tissue polypeptide antigen (TPA), TPS (antigen for M3 antibody), uPAI, VEGF-B, VEGF-C, VEGF-R1, VEGF-R2, and VEGF-R3.

In general, markers can be categorized nonexclusively, and often in overlapping categories as follows: 1. Markers that are detected or detectable by virtue of protein expression or overexpression (detection may occur, e.g. by immunohistochemistry or *in situ* hybridization); 2. Markers that are detected or detectable by virtue of mRNA expression or overexpression (detection may occur, e.g. by differential display techniques); 3. Markers that are detected or detectable by virtue of a post translational change in a protein, e.g. a phosphorylation of the protein, a ubiquitination, a farnesylation, methylation, or other modification to the protein that can be detected, e.g. by antibodies specific to the post translational modification; and 4. Markers may also be detected based on alteration of a gene, for example methylation of the gene, for example, methylation of the retinoic acid receptor beta-2 gene (RARbeta-2), as described in Widschwenedter et al., *J. Natl Cancer Inst.* 2000 92 (10):826-32.

Genes that are overexpressed in breast cancer and can be found by differential display include, e.g. Claudin-7, zinc-alpha-2-glycoprotein, apolipoprotein B, B94, EST (R08988), thrombospondin (THBS1), FGF-1, NGAL-Lipocalin 2, EST (N77731), BS247 (Abbott labs WO 99/22027), AIB-1. Genes that are identifiable by tyrosine phosphorylation changes include, e.g. Erb-B2 and EGFR. Genes that are identifiable by gene methylation include e.g. 14-3-3, SPR1, cyclin D2, GST-pi, and estrogen. Markers that are absent in tumors and are thus termed tumor suppressor markers and when absent or lower than normal levels indicate a tumorogenic condition, include, e.g. mammastatin, maspin, retinoic acid receptor-beta 2, and BRMS1 (a metastasis-suppressor gene). See, *Cancer Res* 2000 60: 2764-2769.

Accordingly, markers such as the following can sought in ductal fluid, e.g. proteins that are overexpressed, mRNA transcripts that are over expressed, and proteins comprising post translational modifications. For example, the following markers can be identified to distinguish a cancer or precancer cell from a normal cell. Proteins that are overexpressed can include e.g. Stromelysin-3, Membrane Type 1 Matrix

Metalloproteinase (MT1-MMP), Matrix Metalloproteinase-3 (MMP-3), Placental Isoferrintin (p43) , Nuclear Matrix Protein (NMP22), NM-200.4 specific antigen, Vascular Endothelial Growth Factor (VEGF), Endoglin (CD105), Telomerase, ErbB-2, ErbB-3, Carcinoembryonic Antigen (CEA), Heat Shock protein-27 (HSP-27), Breast Cancer-specific Gene (BCSG), Plasminogen Activator Inhibitor (PAI-1), Urokinase Plasminogene Activator (uPA), Urokinase Plasminogene Activator Receptor (uPAR), Colony Stimulating Factor-1 (CSF-1), Colony Stimulating Factor-1 receptor (fms), Annexin I, Vasopressin, the CC Chemokine Regulated on Activation Normal T cell Expressed and Secreted (RANTES), 44-3A6 specific antigen, A-80 specific antigen, MUC-1, H23 specific antigen, 83 D4 specific antigen, SP-2 specific antigen, 323/A3 specific antigen, tumor associated antigen-72 (TAG-72) , and MBE6 specific antigen.

Other breast cancer markers detected by any means including e.g. protein expression, mRNA expression, or post translational modification can include e.g. (listed alphabetically) alanine aminopeptidase, alpha 6 integrin, alpha-lactalbumin, AN43, p53, Bcl2-antagonist of cell death (Bad), Bcl2-associated athanogene (BAG-1), Bcl2-antagonist/killer 1 (Bak), Bcl2-associated X protein (Bax), Breast cancer antigen 225 (BCA225), B-cell CLL/lymphoma 2 (Bcl-2), Bcl2-like 1 (Bcl-x), beta 1-6 branched oligosaccharides, beta-2 microglobulin (BMG), Bcl2 related protein A1 (Bfl-1), bone sialoprotein (BSP), CCAAT/enhancer-binding protein liver-enriched inhibitory protein (C/EBPbeta-LIP), Carcinoma Antigen 1 (Ca 1), Carcinoma Antigen 27.29 (CA 27.29), Carcinoma Antigen M26 (CA M26), Carcinoma Antigen M29 (CA M29), Carcinoma Antigen 125 (CA125), Carcinoma Antigen 15.3 (CA15.3), Carcinoma Antigen 195 (CA195), Carcinoma Antigen 19-9 (CA19-9), Carcinoma Antigen 50 (CA50), Carcinoma Antigen 549 (CA549), Cadherin-11, calcitonin receptor (CTR), cathepsin B, cathepsin L, Endoglin (CD105), CD24, CD34 (pan-endothelial marker), CD44, c-met/hepatocyte growth factor receptor, c-myc, cyclooxygenase-1 (Cox-1), cyclooxygenase-2 (Cox-2), caspase-3 (CPP32), Cyclic nucleotide phosphodiesterase, cyclin E, DNA topoisomerase II-alpha, DNA topoisomerase II-beta, EGF, EGF receptor, E-selectin, fast homoarginine-sensitive alkaline phosphatase (FHAP), fatty acid synthase, ferritin, gross cystic disease fluid protein (GCDFP-15/BRST-2), metastasis-associated h-mts1 (S100A4), heat shock cognate protein-73 (hsc73), heat shock protein-70 (hsp70), heat shock protein-90 alpha (hsp90alpha), heat shock protein-90 beta (hsp90beta), inhibitors of differentiation-1 (ID1), inhibitors of differentiation-3 (ID3), interleukin-1 beta, Keratin 8, Keratin 18, Keratin 19, Laminin, Laminin receptor (MLuC5), Leucine Aminopeptidase (LAP), lipid-

bound sialic acid (LSA), Melanoma antigen-1 (MAGE-1), Melanoma antigen-2 (MAGE-2), Melanoma antigen-3 (MAGE-3), Man6-P glycoproteins, Mucin-like carcinoma associated antigen (MCA), myeloid cell leukemia-1 (Mcl-1), metallothionein (MT), mitogen-activated protein kinase phosphatase-1 (MKP-1), Matrix Metalloproteinase-2 (MMP-2), Matrix Metalloproteinase-9 (MMP-9), mammary serum antigen (MSA), breast cancer mucin-2 (MUC-2), breast cancer mucin-3 (MUC-3), breast cancer mucin-6 (MUC-6), Nm23 nucleoside diphosphate kinase, ornithine decarboxylase (ODC), osteopontin (OPN), P114 (MAR binding protein), P120 (a nucleolar proliferation antigen), focal adhesion kinase p125FAK, nuclear autoantigen p330d/CENP-F, plasminogen activator inhibitor-2 (PAI-2), Pepsinogen C, placental alkaline phosphatase (PLAP), Platelet factor 4 (angiogenic marker), protein kinase C (PKC), prostate specific antigen (PSA), pyrimidine nucleoside phosphorylase, ras p21, reduced glutathione (GSH), retinoid X receptor alpha, ribosomal S2 protein, sialyltransferase, Stromelysin-1 (MMP-3), surfactant proteins A, surfactant proteins B, tumor associated antigen-12 (TAG-12), trefoil gene TFF1, trefoil gene TFF3/ITF/hP1.B, Thrombin, Thrombomodulin, thymidine phosphorylase (TP), thymosin beta 15, tissue cytosol ferritins, tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), Vascular Endothelial Growth Factor -B (VEGF-B), Vascular Endothelial Growth Factor-C (VEGF-C), Vascular Endothelial Growth Factor receptor-1(VEGFR1), Vascular Endothelial Growth Factor receptor-2 (VEGFR2), and Vascular Endothelial Growth Factor receptor-3 (VEGFR3).

With respect specifically to nuclear matrix proteins the process is similar as for other markers: the ductal fluid can be examined to determine the presence of a marker comprising an expression product of a gene encoding a nuclear matrix protein. The expression product can comprise a nucleic acid or a polypeptide. The expression product can comprises an RNA. The expression product can comprise a protein or a part of a protein. The nuclear matrix protein can be selected from the group consisting of lamin A, lamin B, lamin C, a peripheral matrix protein, nuclear mitotic spindle apparatus protein (NuMA), topoisomerase II, and an internal nuclear matrix protein. The expression product can be a polypeptide and examining the polypeptide can comprise contacting the polypeptide marker with an antibody that specifically binds a portion of the polypeptide.

The expression product can be a nucleic acid and examining it can comprise detecting the presence of the nucleic acid. Detecting the presence of the nucleic acid can comprise amplifying the nucleic acid, e.g. by PCR. For example, the ductal fluid

can be tested for the presence of NMP66, a nuclear matrix protein specific for breast cancer. The advantage of testing for NMP molecules by retrieval of ductal fluid is that the sensitivity is increased, and the likelihood of early detection of ductal carcinoma *in situ* is increased by measuring a local concentration of the marker and not a concentration derived from the whole body. This advantage is also aided by the opportunity that ductal fluid and cell retrieval via a known and identified duct provides the opportunity to treat the duct or ductal system and not necessarily the breast. This may be important especially where precancer (such as ductal carcinoma *in situ*) is detected, and such detection does not necessarily warrant either a systemic treatment, nor a treatment directed to active, invasive cancer. Detection of breast cancer specific NMPs is described, e.g., in U.S. Patent No. 5,914,238.

Once the ductal fluid is located or isolated, the fluid can be tested for the presence of one or more expression products of genes encoding nuclear matrix proteins (e.g. either an RNA or a polypeptide) in order to evaluate a presence of cancerous or precancerous cells in the duct. Such tests can typically be antibody or nucleic acid amplification tests that are commonly performed in the art of marker detection. The gene products identified can be any nuclear matrix protein gene product, including nuclear matrix gene products specific to malignancy in the breast, and possibly including e.g. lamin A, lamin B, lamin C, a peripheral matrix protein, nuclear mitotic spindle apparatus protein (NuMA), topoisomerase II, or an internal nuclear matrix protein. Assays, kits and methods described in U.S. Patent Nos. 5,965,376, 5,914,238, 5,882,876, 5,858,683, 5,840,503, 5,830,677, 5,783,403, 5,780,596, 5,698,439, 5686,562, or 5,547,928 can be adapted and applied to testing ductal fluid samples.

Once the ductal fluid is analyzed for one or more markers, the fluid may also be analyzed cytologically to determine the cytological status of the ductal epithelial cells and other cells. Cytological assays that can be performed on the cells retrieved from a duct or from nipple aspirate can include e.g. assays described in King *et al, J. Nat'l Cancer Inst* (1983) 71:1115-21, Wrensch *et al.* (1992) *Am. J. Epidem.* 135: 130-141, Papanicolaou *et al*, (1958) *Cancer*, 11:377-409 and Goodson WH & King EB, *Chapter 4: Discharges and Secretions of the Nipple* , THE BREAST: COMPREHENSIVE MANAGEMENT OF BENIGN AND MALIGNANT DISEASES (1998) 2nd Ed. vol 2, Bland & Kirby eds. W.B. Saunders Co, Philadelphia, PA pp. 51-74. For example, as described in Goodson and King (page 60) atypical hyperplasia presents as having cellular abnormalities, increased coarseness of the chromatin, and tendency for more single cells

as well as groups of cells. With regard to carcinoma *in situ*, Papanicolaou *et al*, described cellular abnormalities, e.g. nuclear abnormalities diagnosed by cytology of fluid from nipple secretions containing ductal cells. The cytology of abnormal cells can also be conducted as described in Sartorius *et al* (1977) *J. Natl Cancer Inst* 59: 1073-1080. and King *et al*, (1983) *JNCI* 71(6) 1115-1121. Atypia and carcinoma *in situ* are widely characterized pathologically, as described in Page *et al,* (1998) *Mod Pathol* 11(2): 120-8. The ductal fluid can be analyzed by cytological techniques by placing some of the fluid on a slide with a standard cytological stain using a light microscope. The cells can be studied for atypical growth patterns in individual cells and clusters of cells using published methods, including Mouriquand J, (1993) S Karger Pub, "Diagnosis of Non-Palpable Breast Lesions: Ultrasonographically Controlled Fine-Needle Aspiration: Diagnostic and Prognostic Implications of Cytology" (ISBN 3805557477); Kline TS and IK, Pub Igaku-Shoin Medical ""Breast: Guides to Clinical Aspiration Biopsy" (LSBN 0896401596; Masood, *American Society of Clinical Pathology*: Nov. 199S, "Cytopathology of the Breast" ISBN 0891893806; and Feldman PS, *American Society of Clinical Pathology*, Nov. 1984, "Fine Needle Aspiration Cytology and Its Clinical Applications: Breast and Lung" ISBN 0891891846.

Other references that discuss cytological analysis and which give guidance to an analysis of ductal epithelial cells derived from ductal fluid include Silverman et al, (Can FNA biopsy separate atypical hyperplasia, carcinoma *in situ*, and invasive carcinoma of the breast?: Cytomorphologic criteria and limitations in diagnosis, Diagnostic Cytopathology) 9(6):713-28, 1993; Masood et al, (Immunohistochemical differentiation of atypical hyperplasia vs. carcinoma *in situ* of the breast) *Cancer Detection & Prevention*. 16(4):225-35, 1992; Masood et al, (Cytologic differentiation between proliferative and nonproliferative breast disease in mammographically guided fine-needle aspirates) *Diagnostic Cytopathology*.7(6):581-90, 1991; Masood S., (Occult breast lesions and aspiration biopsy: a new challenge) Diagnostic Cytopathology. 9(6):613-4, 1993; Masood S., (Prognostic factors in breast cancer: use of cytologic preparations) *Diagnostic Cytopathology*. 13(5):388-95, 1995; Novak and Masood, (Nuclear grooves in fine-needle aspiration biopsies of breast lesions: do they have any significance?) *Diagnostic Cytopathology*. 18(5):333-7, 1998; Sidawy *et al,* (Interobserver variability in the classification of proliferative breast lesions by fine-needle aspiration: results of the Papanicolaou Society of Cytopathology Study) *Diagnostic Cytopathology*. 18(2):150-65, 1998; Masood *et al*, (Automation in cytology: a survey conducted by the

New Technology Task Force, Papanicolaou Society of Cytopathology) *Diagnostic Cytopathology*. 18(1):47-55, 1998; and Frykberg and Masood Copeland EM 3d. Bland KI., (Ductal carcinoma *in situ* of the breast) *Surgery, Gynecology & Obstetrics* 177(4):425-40, 1993.

As discussed, the cells collected can comprise ductal epithelial cells and the ductal fluid collected can comprise molecular and cellular material. The collected cells and fluid and fluid components can be analyzed, e.g. as described or suggested herein. Fluid collected from the milk ducts, can include constituents of biological fluids, e.g. those typically found in breast duct fluid, e.g. water, cells, cellular markers, molecular markers, nucleic acids, proteins, cellular debris, salts, particles or organic molecules. These constituents can be analyzed by any appropriate method depending on the marker and the diagnostic purpose. In addition, any of the cells of the duct can be analyzed for morphological abnormalities in cell components, including, e.g. morphological abnormalities of the nucleus, cytoplasm, Golgi apparatus or other parts of a cell. Cell morphology can serve to establish whether the ductal epithelial cells are normal (i.e. not precancerous or cancerous or having another noncancerous abnormality), precancerous (i.e. comprising hyperplasia, atypical ductal hyperplasia (ADH) or low grade ductal carcinoma *in situ* (LG-DCIS)) or cancerous (i.e. comprising high grade ductal carcinoma *in situ* (HG-DCIS), or invasive carcinoma). Analysis of cell contents may serve to establish similar staging as established by morphology, capturing generally a progression of a precancerous or cancerous condition in the cells.

Once the ductal fluid sample is retrieved from the breast it is examined for the presence of a marker such as, for example a protein, a polypeptide, a peptide, a nucleic acid, a polynucleotide, an mRNA, a small organic molecule, a lipid, a fat, a glycoprotein, a glycopeptide, a carbohydrate, an oligosaccharide, and a chromosomal abnormality, a whole cell having a marker molecule, a particle, a secreted molecule, an intracellular molecule, and a complex of a plurality of molecules as described above. In addition, the marker may be capable of differentiating between any two of cytological categories consisting of normal, abnormal, hyperplasia, atypia, ductal carcinoma, ductal carcinoma in situ (DCIS), ductal carcinoma in situ - low grade (DCIS-LG), ductal carcinoma in situ - high grade (DCIS-HG), invasive carcinoma, atypical mild changes, atypical marked changes, atypical ductal hyperplasia (ADH), insufficient cellular material for diagnosis, and sufficient cellular material for diagnosis. These categories classify the epithelial cells cytologically, and these classifications may indicate either cancer or its

precursors, or absence of cancer indicia. The marker may be capable of differentiating between any two of cytological categories consisting of normal, abnormal, hyperplasia, atypia, ductal carcinoma, ductal carcinoma in situ (DCIS), ductal carcinoma in situ - low grade (DCIS-LG), ductal carcinoma in situ - high grade (DCIS-HG), invasive carcinoma, atypical mild changes, atypical marked changes, atypical ductal hyperplasia (ADH), insufficient cellular material for diagnosis, and sufficient cellular material for diagnosis.

For example, the number of epithelial cells in ductal lavage samples may range from none to several thousand. At least ten epithelial cells are required to designate a sample as adequate. Benign duct cells may be present singly, in monolayer sheets, or in tight clusters, using one to two cell layers thick. The cells are small with small nuclei (in a range from about 8 to about 12 μm in diameter). The nuclear to cytoplasmic ration may appear to be high depending on the orientation of the cells in clusters. Single benign duct cells are often difficult to identify, often appearing similar to surrounding lymphocytes or histiocytes. Duct cells may be recognized by the columnar shape of their cytoplasm, or by the presence of discreet small vacuoles in the cytoplasm. The smooth, discrete cytoplasmic border may also help to distinguish duct cells. Benign duct cells are more easily recognized when they occur in groupings. Cohesive groups, as opposed to looser clusters, are more suggestive of epithelial origin. Benign groups are one or two cell layers in thickness, and are composed of cells which are uniform in size. The cell nuclei are also uniform in size, and are regularly round to oval in shape. Markers that may be identified in addition to cytological notations, may assist a diagnosis by confirming a cytological reading and or adding additional information to any noteworthy subcategory within the category of benign.

The cytological category including atypical epithelial cells, with mild changes, includes duct cells from proliferative conditions including hyperplasia. The cells may occur singly, in cohesive multilayered and complex groups, and in monolayers. The groups may show an increase in the number of cell layers, which can be appreciated by focusing through the groups. Duct groupings also may show increased overlap, with nuclear crowding. The cells may be minimally enlarged, and may show moderate increases in nuclear size, in a range from 12 to 16 μm in diameter. Slight anisonucleosis may be present among cells in groups. Nucleoli are often present. Markers found in the ductal fluid may assist to identify atypical cells or atypical cells with mild changes, or may confirm such cytological identification.

Atypical cells can also include atypical cells with marked changes. More marked changes are often associated with atypical hyperplasia and low grade ductal carcinoma in situ (DCIS). Enlarged duct cells may be present, showing more marked nuclear increase and variation in size and shape. Single cells are enlarged, with the cytoplasm in some cases abundant, nuclear-to-cytoplasmic rations may actually appear decreased. Chromatin may appear coarse, with mild abnormality in distribution. Nucleoli may be larger, multiple, and more prominent. Nuclei in groups may appear to be overlapping. Mitotic figures may be seen. Markers found in the ductal fluid may assist to identify atypical cells with marked changes, or may confirm such cytological identification.

Malignant epithelial cells include duct cells from high grade breast carcinoma and exhibit common features of malignancy. More single cells are present, as cell cohesiveness is lost. Loose clusters of epithelial cells are present, along with the more usual tight groups of cells. Cell and nuclear enlargement may be marked. High nuclear to cytoplasmic ratios may be present in some cases. However, some high grade specimens often have lots of cytoplasm in a portion of the tumor cells, resulting in low or variable nuclear-to-cytoplasmic ratios. Nuclear membranes are often irregular, and chromatin is clumped, hyperchromatic, and unevenly dispersed. Nucleoli are often large and conspicuous and may be multiple. Marked variation among the cells can be seen in terms of cell and nuclear size. Accompanying these changes is often a background of necrotic debris. Microcalcifications may be seen in the background material. These may appear as dense material with smooth borders and concentric layers, or may be dystrophic, amorphous in nature. Markers found in the ductal fluid may assist to identify malignant cells, aspects of malignant indicia, or may confirm such cytological identification. Markers may also help to stage the malignancy or provide other valuable information which might aid in directing a detailed diagnosis and/or viable treatment options.

Other cytological criteria and processes related to ductal fluid analysis are described in Barret et al, Acta Cytol 1976;20:174-180; Goodson et al, Discharges and Secretions of the Nipple, THE BREAST: COMPREHENSIVE MANAGEMENT OF BENIGN AND MALIGNANT DISEASES, Second Edition, Vol. 1, Chapter 4, page 1; King et al, Cytometry 1984; 5: 124-130; King et al, A.J.C.P. 1975; 64: 739-748; King et al, A.J.C.P. 1975; 64: 728-738; King et al, Cytopathology of Abnormal Mammary Duct Epithelium, Prevention and Detection of Cancer, Part II, Detection, vol 2 Cancer

detection in specific sites, 1976; King et al, J Natl Cancer Inst, 1983; 71: 1115-1121; Kjellgren et al, Acta Cytol 1964; 8: 216-217; Masood et al, The Breast Journal 1999; 5:1-2; Papanicolaou et al, Cancer 1958; 377-409; Petrakis et al, Cancer Epidemiology, Biomarkers and Prevention 1993; 2:3-10; Ringrose et al, Acta Cytol 1966; 10:373-375; Sartorius et al, NCI 1977; 59:1073-1080; Sauter et al, British J. Cancer 1997; 76(4):494-501; Wrensch et al, Amer J. Epid. 1992; 135: 130-141.

The invention also provides systems for diagnosing breast cancer or precancer comprising a tool to retrieve ductal fluid from a breast duct and instructions for use to determine the presence of a marker identified. The systems can comprise kits which include a ductal access tool, for example in order to retrieve the ductal fluid, e.g. especially where it is preferred that the ductal fluid be identified as coming from a particular duct (so that the duct can be accessed later for treatment or further monitoring). The instructions in the kits can include directions according to the methods of identifying breast cancer or precancer described herein, and including any marker or markers or marker classification group or groups that are described herein. The kit or system can include assay reagents for detecting the marker or markers. The kit may comprise a panel for testing a plurality of markers either simultaneously or sequentially, or some practical combination of testing modalities. The kit can also include indexes and parameters for making a diagnosis, depending on the marker or markers. The kit can include a container for the kit contents.

The following table supplies some exemplary markers and marker categories and publications that identify particular identification methods for that category of marker and/or for the specific marker as well.

TABLE I

#	CATEGORY	SPECIFIC EXAMPLES	NOTES (further description; marker forms and identification contexts; assays)
1	Integral membrane protein	Claudin (e.g. claudins 1, 2, or 3)	Kubota et al, Curr Biol. 1999 ; 9(18):1035-8 Furuse et al, J Cell Biol. 1998; 141(7):1539-50
2	Glycoprotein Hormone responsive protein Cytosolic protein	Zinc-alpha-2-glycoprotein	Lopez-Boado et al, Breast Cancer Res. Treat 1994; 29(3):247-58 Diez-Itza et al, Eur. J. Cancer 1993; 29A(9):1256-60 Chaubert and Hurlimann, Arch Pathol Lab Med 1992; 116(11):1181-8
3	Aspartyl proteinase Differentiation associated protein	Gross cystic disease fluid protein-15 kD (GCDFP-15)	Chaubert and Hurlimann, Arch Pathol Lab Med 1992; 116(11):1181-8 Caputo et al, J Biol Chem, 2000; 275(11):7935-41

#	CATEGORY	SPECIFIC EXAMPLES	NOTES (further description; marker forms and identification contexts; assays)
4	Lipoprotein	Apolipoprotein	Lane et al, Breast Cancer Res Treat 1995; 34(2):161-9
5	Peptide	CD36-binding peptide	Carron et al, Biochem Biophys Res Comm 2000 270(3):1124-1127
6	Ligand Extracellular matrix protein	Thrombospondin-1	Carron et al, Biochem Biophys Res Comm 2000 270(3):1124-1127 Murphy-Ullrich and Poczatek, Cytokine Growth Factor Rev 2000; 11(1-2):59-69
7	Growth factor	Fibroblast growth factor	Fernig et al, Biochem Biophys Res Comm 2000; 267(3):70-6
8	Lipocalin	Neu-related lipocalin/neutophil gelatinase-associated lipocalin (NRL/NGAL)	Zerega et al, Eur J Cell Biol 2000; 79(3):165-72
9	Hormone responsive protein	Amplified in breast cancer -1 (AIB-1)	Eng et al, J Biol Chem 1998; 273(43):28371-7
10	Hormone responsive protein	Transcriptional intermediary factor - 1 and –2 (TIF-1 &-2)	Eng et al, J Biol Chem 1998; 273(43):28371-7
11	Transferase	Glutathione S-transferase pi (GST-pi)	Huang et al, Oncol Rep 2000; 7(3):609-13
12	Differentiation associated protein	SPR-1	Anisowicz et al, Mol Med 1999;5(8):526-41
13	Differentiation associated protein Activator of tyrosine and tryptophan hydroxylase	HME-1 (25kd) 14-3-3 sigma protein stratifin	Prasad et al, Cell Growth Differ 1992;3(8):507-13 USPN 5,776,676 (nucleic acid) USPN 4,707,438 (immunoassay) Isobe et al, J. Mol Biol 1991 217(1):125-32 USPN 5,597,719 (Raf interaction) USPN 5,424,191 (polypeptide) 14-3-3 sigma accession no.: AFO29082 Laronga et al, J Biol Chem, 2000: April 14 (no page numbers available)
14	Proto-oncogene Cyclin	Cyclin D1	Bukholm et al, Virchows Arch 1998;433(3):223-8 Sweeney et al, Oncogene 1997; 14(11):1329-40 Buckley et al, Oncogene 1993:8(8):2127-33 Weinstat-Saslow et al, Nat Med 1995;1(12):1257-60 Barnes and Gillett, Breast Cancer Res Treat 1998; 52(1-3):1-15 Reed et al, Virchows Arch 1999; 435(2); 116-24
15	Growth factor	Vascular endothelial growth factor (VEGF) Vascular permiability factor (VPF)	Toi et al, Clinical Cancer Res. 1995; 1:961-964 Lichtenbeld et al, Int J Cancer 1998; 77(3):445-9 Guidi et al, Cancer 1997; 80(10); 1945-53 Locopo et al, Breast Cancer Res Treat 1998; 52(1-3):159-73 Wright et al, Exp Mol Pathol 1997; 64(1):41-51 Yoshiji et al, Cancer Res 1996; 56(9); 2013-6 Brown et al, Human Pathol 1995; 26(1):86-91

Table I (cont'd)

#	CATEGORY	SPECIFIC EXAMPLES	NOTES (further description: marker forms and identification contexts; assays)
16	Receptor	Vascular endothelial growth factor receptor (VEGF-R) flt-1	Yoshiji et al, Cancer Res 1996; 56(9); 2013-6
17	Proteolytic factor	Cathepsins D and L	Harbeck et al, Int J Markers 2000 15(1):79-83 Ioachim et al, Anticancer Res 1998; 18(3A):1665-70
18	Tumor suppressor Angiogenesis inhibitor	Maspin	Domann et al, Int J Cancer 2000; 85(6):805-10 USPN 5,470,970 Zou et al, J Biol Science, 2000; 275(9):6051-4 Zhang et al, Nat Med 2000; 6(2):196-9
19	Receptor Tumor necrosis family Apoptosis pathway protein Cell death protein	Fas Apo-1 CD95	Mottolese et al, Int J Cancer 2000 89(2):127-32 Reimer et al, Cancer Res 2000;60(4):822-8
20	Ligand Tumor necrosis family Apoptosis pathway protein Cell death protein	Fas ligand (fasL)	Mottolese et al, Int J Cancer 2000 89(2):127-32 Reimer et al, Cancer Res 2000;60(4):822-8
21	Inhibitor	Tissue inhibitor of metalloproteinase 1 and 2 (TIMP-1, TIMP-2)	Brummer et al, Virchows Arch 1999; 435(6):566-73 Remacle et al, Int J Cancer 2000: 89(2):118-21 Luparello et al, Breast Cancer Res Treat 1999; 54(3):235-44
22	Chemokine	CC chemokine regulated on activation normal T cell expressed and secreted (RANTES)	Luboshits et al, Cancer Res. 1999; 59(18):4681-7 Prest et al, Clin Exp Metastasis, 1999;17(5):389-96 Luboshits et al, Cancer Res. 1999; 59:4681-4687
23	Chemokine	Macrophage inflammatory protein 1alpha and 2 beta (MIP-1alpha, MIP-2beta)	Tedla et al, Cytokine 1999; 11(7):531-40 Prest et al, Clin Exp Metastasis, 1999;17(5):389-96
24	Cell adhesion molecule	E-cadherin and N-cadherin	Hazan et al, J Cell Biol 2000;148(4):779-90 Boterberg et al, Cell Adhes Comm, 2000; 7(4):299-310
25	Basement membrane protein Antibody	LH39 (antibody recognizing the lamina lucida of mature small veins and capillaries	Kakolyris et al, Br J Cancer 2000; 82(4):844-51
26	Basement membrane protein	Laminin	Sidhom and Imam, Int J Clin Lab Res, 1999; 29(1):26-9 Ioachim et al, Anticancer Res 1998; 18(3A):1665-70
27	Cell adhesion molecule	Integrin beta 1	Berry et al, Eur J Surg Oncol, 2000; 26(1):25-9
28	Cell adhesion molecule	E-selectin	Hebbar et al, Int J Biol Markers, 2000; 15(1):15-21
29		Catenin (E, alpha, beta, gamma)	Bukholm et al, J Pathol, 2000; 190(1): 15-9
30	Receptor	Thrombin receptor	Henrikson et al, Br J Cancer, 1999; 79(3-4):401-6
31	Protease	Urokinase plasminogen activator (uPA)	Stephens et al, Breast Cancer Res Treat, 1998; 52(1-3): 99-111
32	Serpin	Serine protease inhibitors (SERPINS) – alpha-1-antichymotrypsin, alpha-1-antitrypsin, alpha2-	Wojtukiewicz et al, Haemostasis 1998; 28(1):7-13

		macroglobulin, antithrombinIII, C1 inhbitor, alpha2-antiplasmin	
33	Tumor suppressor Tissue specific inhibitor	Mammastatin (polypeptides 47kD and 65 kD)	Ervin et al, Science 1989; 244(4912):1585-7
34	Protein Nucleic Acid	Cytokeratin	Bae et al, J Korean Med Sci 2000; 15(2):194-8
35	Sialic acid	Lipid bound sialic acid (LSA)	Raval et al, Int J Biol Markers, 1997; 12(2):61-7 Romppanen et al, Anticancer Res, 1997; 17(2B):1249-53
36	DNase	Alkaline DNase (ADA)	Raval et al, Int J Biol Markers, 1997; 12(2):61-7
37	Ribonucleoprotein Enzyme	Telomerase	Mokbel et al, Eur J Surg Oncol 2000; 26(1):30-3
38	Serine protease	Prostate specific antigen (PSA)	Black et al, Breast Cancer Res Treat, 2000; 59(1):1-14 Black et al, Clin Cancer Res, 2000; 6(2):467-73
39	Antibody	Antibody specific for myosin smooth muscle heavy chain (SM1, SM2); Antibody specific for myosin non-muscle (NM-MyHC)	Chiavegato et al, Virchows Arch 1995; 426(1):77-86
40	Proliferation associated antigen	Endoglin (EDG, CD105)	Matsuno et al, Clin Cancer Res, 1999; 5(2):371-82

Table I (cont'd)

#	CATEGORY	SPECIFIC EXAMPLES	NOTES (further description; marker forms and identification contexts; assays)
41	Nuclear protein	108kD, 53kD, 48kD nuclear polypeptides	Brys et al, Cytobios 2000; 101(397):87-94
42	Cytoplasmic protein	36kD cytoplasmic polypeptide	Brys et al, Cytobios 2000; 101(397):87-94
43	Transforming growth factor alpha family of proteins	Transforming growth factor alpha (TGF-alpha)	Humphreys and Hennighausen, Oncogene 2000; 19(8) Bourhis et al, Int J Cancer 1997; 71(1):42-8 Yoshiji et al, Cancer Res 1996; 56(9); 2013-6
44	Transforming growth factor beta family of proteins	Transforming growth factor beta (TGF-beta)	Yoshiji et al, Cancer Res 1996; 56(9); 2013-6 Pawlina et al, Mol Cell Endocrinol 1990; 72(1):55-61
45	Protein factor Hormone responsive protein	Colony stimulating factor 1 (CSF-1) M-CSF	Sapi et al, Proc Soc Exp Biol Med 1999:220(1):1-8 Tang et al, J. of Cellular Biochemistry 1992; 50:350-356 Sapi et al, J Soc Gynecol Investig 1998; 5(2):94-101
46	Receptor Hormonre responsive protein	Colony stimulating factor receptor (CSF-1R)	Sapi et al, Proc Soc Exp Biol Med 1999:220(1):1-8 Sapi et al, Cancer Res 1999; 59(21):5578-85 Maher et al, Clin Cancer Res 1998; 4(8):1851-6 Sapi et al, Oncogene 1995; 10(3): 529-42 Tang et al, J. of Cellular Biochemistry 1992; 50:350-356 Sapi et al, J Soc Gynecol Investig 1998; 5(2):94-101 Flick et al, Oncogene 1997; 14(21):2553-61
47	Proto-oncogene	c-fms	Sapi et al, Proc Soc Exp Biol Med 1999:220(1):1-8 Sapi et al, Cancer Res 1999; 59(21):5578-85 Maher et al, Clin Cancer Res 1998; 4(8):1851-6 Sapi et al, Oncogene 1995; 10(3): 529-42
48	Phospholipid binding protein Actin binding protein Calcium binding protein	Annexin-1	Pencil and Toth, Clin Exp Metastasis 1998; 16(2):113-21 Ahn et al, Clin Exp Metastasis 1997;15(2):151-6
49	Receptor G-protein linked receptor	Vasopressin and Oxytocin receptors	Zingg, Baillieres Clin Endocrinol Metab 1996:10(1):75-96
50		Vasopressin: arginine vasopressin (VP), provasopression (ProVP), vaopressin-associated human glycopepeptide (VAG)	North et al, Breast Cancer Res Treat 1995; 34(3): 229-5 North et al, Breast Cancer Res Treat 1995; 34:229-235
51		Oxytocin (OT), oxytocin associated human neurophysin (OT-HNP)	North et al, Breast Cancer Res Treat 1995; 34(3): 229-5
52	Peptidase	Alanine aminopeptidase (AAP)	Severini et al, Cancer Biochem Biophys 1991;12(3):199-204
53	Antigen	Tissue polypeptide antigen (TPA or TPS) Antigen recognized by M3 antibody	Severini et al, Cancer Biochem Biophys 1991;12(3):199-204 Aydiner et al, Acta Oncol 1994; 33(2): 181-6
54	Lactalbumin	Alpha-lactalbumin	Simickova et al, Neoplasma 1991; 38(4):407-13
55	Bcl2 family member Proto-oncogene	Bcl-2	Schorr et al, J Mammary Gland Biol Neoplasia, 1999; 4(2): 153-64 Knowlton et al, J Surg Res, 1998; 76(1): 22-6

			Veronese et al, Int J Cancer, 1998; 79(1): 13-8 Olopade et al. Cancer J Sci Am, 1997; 3(4): 230-7 Zhang, et al, Clin Cancer Res 1997; 3(12): 2329-35
56	Bcl2 family member	Bax	Schorr et al, J Mammary Gland Biol Neoplasia, 1999; 4(2): 153-64 Knowlton et al, J Surg Res, 1998; 76(1): 22-6 Veronese et al, Int J Cancer, 1998; 79(1): 13-8 Olopade et al, Cancer J Sci Am, 1997; 3(4): 230-7
57	CCAAT/enhancer binding protein family (C/EBP)	C/EBP	Rosen et al, Biochem Soc Symp 1998; 63:101-13
58	Bone matrix protein	Bone sialoprotein (BSP)	Castronovo and Bellahcene, Int J Oncol 1998; 12(2): 305-8
59	Metastasis associated protein Carbohydrate antigen	CA-15-3	Aydiner et al, Acta Oncol 1994; 33(2): 181-6 Guarner et al, Arch Med Res 1997; 28(4):523-6
60	Metastasis associated protein	Beta-2 microglobulin (BMG)	Aydiner et al, Acta Oncol 1994; 33(2): 181-6
61	Metastasis associated protein	ferritin	Aydiner et al, Acta Oncol 1994; 33(2): 181-6
62	Glycoprotein	P-glycoprotein (MDR1 or MRP gene expression product)	Sikic, Ann Oncol 1999; 10 suppl 6:149-53 Kroger et al, Cancer Treat Rev 1999; 25(5):279-91
63	CCAAT/enhancer binding protein family (C/EBP)	ICBP90 (89, 758 kD)	Hopfner et al, Cancer Res 2000; 60(1):121-8

Table I (cont'd)

#	CATEGORY	SPECIFIC EXAMPLES	NOTES (further description: marker forms and identification contexts: assays)
64	Enzyme	Aromatase (CYP19)	Brueggemeier et al, Cancer Lett 1999; 140(1-2): 27-35
65	Enzyme	Cyclo-oxygenase 1, 2 (COX-1, COX-2) Prostoglandin endoperoxide synthase	Brueggemeier et al, Cancer Lett 1999; 140(1-2): 27-35 Liu and Rose, Cancer Res 1996; 56(22):5125-7
66	Antigen	PGE2	Brueggemeier et al, Cancer Lett 1999; 140(1-2): 27-35
67	Hormone responsive protein	Hormone induced gene-1 (HI-1)	Russo and Russo, J Cell Biochem Suppl 2000; 34:1-6
68	Heat shock protein	BAG-1 (Hsp70/Hsc70)	Krajewski et al, Endocr Relat Cancer 1999; 6(1): 29-40
69	Bcl2 family of proteins	Bcl-X(L)	Krajewski et al, Endocr Relat Cancer 1999; 6(1): 29-40
70	Caspase	Caspase-3	Krajewski et al, Endocr Relat Cancer 1999; 6(1): 29-40
71	Antigen Tumor rejection antigen	Melanoma associated antigen-1, -3 (MAGE-1, -3) MZ2-E (antigen) MZ2-D (antigen)	Fujie et al, Ann Oncol 1997; 8(4): 369-72 Toso et al, Cancer Res 1996;56(1): 16-20 Brassaur et al, Int J Cancer 1992;52(5):839-41 Gaugler et al, J Exp Med 1994; 179(3): 921-30
72	Antigen Tumor rejection antigen	SART-1 (800kD antigen)	Kawamoto et al, Int J Cancer 1999; 80(1):64-7
73	Inhibitor	P16 (INK4, MTS-1) Inhibitor or cyclin D-CDK4 complex	Jaffrain-Rea et al, Clin Endo 1999; 51: 317-325
74	Nuclein	Breast cancer specific gene-1 (BCSG-1) gamma-synuclein (SNCG) SNC-gamma	Ji et al, Cancer Res 1997; 57(4):759-64 Lavedan et al, Hum Genet 1998:103(1):106-12 Jia et al, Cancer Res. 1999; 59(3):742-7 Ji et al, Cancer Res 1997; 57:759-764
75	Peptide	Fibrinogen degredation peptide	WO 98/55872
76	Gap junction protein	Connexin 26	Jamieson et al, J Pathol 1998; 184(1):37-43
77	Gap junction protein	Connexin 43	Jamieson et al, J Pathol 1998; 184(1):37-43
78		Fibronectin	Ioachim et al, Anticancer Res 1998; 18(3A):1665-70
79	Inhibitor	Relaxin	Pawlina et al, Mol Cell Endocrinol 1990; 72(1):55-61
80	Growth factor	Fibroblast growth factor - basic (FGFb)	Yoshiji et al, Cancer Res 1996; 56(9); 2013-6
81	Antigen Dedifferentiation marker	Carcinoembryonic antigen (CEA)	Kuhajda et al, Cancer 1983; 52:1257-1264 Schmitt and Andrade, J Clin Pathol 1995;48(1):53-6 Mangili et al, Cancer 1996;78(11):2334-9
82	Differentiation marker	Human milk fat globulin (HMFG)	DePotter et al, Pathol Res Pract 1988; 183(3):271-6
83	Phosphoprotein Tumor suppressor	p53	Poller et al, Br J Cancer 1992; 66:583-588 Poller et al, Hum Pathol 1993;24(5): 463-8 Zhang, et al, Clin Cancer Res 1997; 3(12): 2329-35 Schmitt et al, J Pathol 1995:176(3):233-41 Rajan et al, 1997; 42(3):283-90 Rudas et al, Eur J Cancer 1997;33(1):39-44 Lisboa et al, Virchows Arch 1997; 431(6):375-81 Done et al, Cancer Res. 1998; 58(4):785-9 Naidu et al, Anticancer Res. 1998; 18(1A):65-70

			Jerry et al, Oncogene 2000; 19(8):1052-8 Ioachim et al, Anticancer Res 1998; 18(3A):1665-70 Midulla et al, Anticancer Res 1999; 19(5B):4033-7
84	Receptor	Epidermal growth factor receptor	Poller et al, Br J Cancer 1992; 66:583-588 Poller et al, Hum Pathol 1993;24(5): 463-8 Ioachim et al, Anticancer Res 1998; 18(3A):1665-70
85	Oncogene Oncoprotein	c-erbB-2	Poller et al, Br J Cancer 1992; 66:583-588 Poller et al, Hum Pathol 1993;24(5): 463-8 Lodata et al, Mod Pathol. 1990; 3(4):449-54 Ioachim et al, Anticancer Res 1998; 18(3A):1665-70 Zaretsky et al, FEBS 1990; 265(1-2): 46-50 Mangili et al, Cancer 1996: 78(11): 2334-9
86	Oncogene Oncoprotein	c-erbB-3	Naidu et al, Br. J. Cancer 1998; 78(10):1385-90
87	Antigen	Her-2/neu	Allred et al, Hum Pathol. 1992; 23(9):974-9 Storm et al, Ann Surg Oncol 1995; 2(1):43-8
88	Heat shock protein	Heat shock protein – 27	Storm et al, Ann Surg Oncol 1995; 2(1):43-8
89	Protease	Urokinase plasminogen activator (uPA)	Kennedy et al, British J. of Cancer 1998; 77(10): 1638-1641 Bianchi et al, Cancer Res. 1994; 54:861-866 Grondahl et al, Cancer Res. 1993; 53:2513-2521

Table I (cont'd)

#	CATEGORY	SPECIFIC EXAMPLES	NOTES (further description; marker forms and identification contexts; assays)
90	Receptor	Urokinase plasminogen activator receptor (uPAR)	Bianchi et al, Cancer Res. 1994; 54:861-866
91	Proteolytic factor inhibitor	Urokinase-type plasminogen activator (uPA) inhibitor type 1 (PAI-1)	Grondahl et al, Cancer Res. 1993; 53:2513-2521 Harbeck et al, Int J Markers 2000 15(1):79-83 Bianchi et al, Int J. Cancer 1995; 60(5):597-603 Harbeck et al, Breast Cancer Res Treat 1999; 54(2):147-57
92	Whole cell having a marker molecule	Oncofetal ferritin bearing lymphocytes (FBL)	Moroz et al, Cancer 1989; 64(3):691-7
93	Ferritin Antigen	Oncofetal ferritin Placental isoferritin (p43) (PLF)	Moroz et al, Cancer 1989; 64(3):691-7 Auberbach et al, Abstracts and Proceedings from 10th European Cancer Conference 9-12-99 to 9-16-99, Vienna Austria; abstract 589 Rosen et al, Breast Cancer Res Treat 1992; 24(1):17-26 Reinerova et al, Neoplasma 1996:43(6):363-6 Rosen et al, Cancer Lett 1991; 59(2):145-51 Stierer et al, Breast Cancer Res Treat 1991; 19(3):283-8 Rosen et al, Cancer Lett 1992; 67(1):35-45
94	Protein	Type IV collagen	Ioachim et al, Anticancer Res 1998; 18(3A):1665-70
95	Proliferation associated antigen	Ki-67	Ioachim et al, Anticancer Res 1998; 18(3A):1665-70
96	Proliferation associated antigen	PCNA	Ioachim et al, Anticancer Res 1998; 18(3A):1665-70
97	Zinc endoprotease Collagenases Gelatinases Stromelysins Matrilysin Metalloelastase	Matrix metalloproteinase 1, 2, and 3 (MMP-1, MMP-2, MMP-3) Collagenases (MMP-1, MMP-8, MMP-13) Gelatinases (MMP-2, MMP-9) Stromelysins (MMP-3, MMP-10) Matrilysin (MMP-7) Metalloelastase (MMP-12) MMP-14	Brummer et al ,Virchows Arch 1999; 435(6):566-73 Jpn J Cancer Res 1999; 90:516-522 Polette et al, Clin Exp Metastasis 1997; 15:157-163 Ueno et al, Cancer Res 1997; 57: 2055-2060 Nakopoulou et al, Hum Pathol. 1999; 30(4):436-442 Lee et al, Clin Exp Metastasis 1996 14(6):512-9 Polette et al, Invasion Metastasis 1993;13(1):31-7 Ioachim et al, Anticancer Res 1998; 18(3A):1665-70 Su et al, Hybridoma 1995; 14(4):383-390 Rha et al, Breast Cancer Res Treat 1997; 43(2):175-81
98	Stromelysin	Stromelysin-3 (ST3) Stromelysin-3 (MMP-11)	Hahnel et al, Int J Cancer 1993; 55(5):771-4 Hahnel et al, Int. J Cancer 1994; 58(2):157-60 Kawami et al, Anticancer Res 1993; 13(6A):2319-23 Brummer et al ,Virchows Arch 1999; 435(6):566-73 Jpn J Cancer Res 1999; 90:516-522 Polette et al, Clin Exp Metastasis 1997; 15:157-163 Ueno et al, Cancer Res 1997; 57: 2055-2060 Nakopoulou et al, Hum Pathol. 1999; 30(4):436-442 Lee et al, Clin Exp Metastasis 1996 14(6):512-9 Polette et al, Invasion Metastasis 1993;13(1):31-7 Ioachim et al, Anticancer Res 1998; 18(3A):1665-70

			Engel et al, Int J Cancer 1994: 58(6):830-5 Su et al, Hybridoma 1995; 14(4):383-390 Santavicca et al, Int J Cancer 1995; 64:336-341 Basset et al, Nature 1990: 348:699-704
99	Zinc endoprotease collagenase	MMP-2 72 kD type IV collagenase gelatinase	Talvensaari-Mattila et al, Cancer 1998; 83(6):1153-62 Kawami et al, Anticancer Res 1993; 13(6A):2319-23 Su et al, Hybridoma 1995; 14(4):383-390
100	Kinase	Nm23 Nucleoside diphosphate kinase	Hahnel et al, Int. J Cancer 1994; 58(2):157-60
101	Antibody Antigen	Mab 44-3A6 (detecting a 40 kD cell surface protein on adenocarcinomas)	Duda et al, Tumor Biol 1991; 12:254-260
102	Antibody Antigen	Mab A-80 (detecting mucin type glycoprotein; tumor associated cytoplasmic mucin-type glycoprotein) MM 1-80 Polymorphic epithelial mucin (PEM)	Shin et al, APMIS 1989; 97(12):1053-260 Eriksson et al, Human Pathol 1992; 23(12): 1366-1372 Castagna et al, Path Res Pract. 1992; 188: 1002-1008

Table I (cont'd)

#	CATEGORY	SPECIFIC EXAMPLES	NOTES (further description; marker forms and identification contexts; assays)
103	Antibody Antigen	Mab to DF3 (detecting tumor associated antigen 290 kD cell surface protein in breast carcinoma cells; CA15.3 antigen)	Kufe et al, Hybridoma 1984; 3(3):223-32 Ohuchi et al, JNCI (1987); 79(1):109-115 Szpak et al, Acta Cytol. 1984; 28(4): 356-67
104	Antibody Antigen	H23 breast tumor associated antigen *Gene 17.5*	Zaretsky et al, FEBS 1990; 265(1-2): 46-50 Keydar et al, Proc. Natl. Acad Sci USA 1989; 86:1362-1366 Stein et al, Int J Cancer 1991; 47(2):163-9
105	Protein	pS2	Zaretsky et al, FEBS 1990; 265(1-2): 46-50
106	Antibody Antigen	Antibody B72.3 Tumor associated glycoprotein-72 (TAG-72) Oncofegal antigen tumor associated glycoprotein – 72	Stein et al, Int J Cancer 1991; 47(2):163-9 Thor et al, Cancer Res 1986; 46: 3118-3124 Prey et al, Human Pathol 1991; 22(6): 598-602 Contegiacomo et al, Eur J Cancer 1994; 30A(6): 813-820 Shousha et al, J Clin Pathol. 1990; 43(12): 1026-8 Mangili et al, Cancer 1996: 78(11): 2334-9 Szpak et al, Acta Cytol. 1984; 28(4): 356-67 Muraro et al, Cancer Res 1988; 48(16): 4588-96 Stein et al, Int J Cancer 1991; 47(2): 163-9 Lottich et al, Breast Cancer Res Treat 1985; 6(1): 49-56 Nuti et al, Int J Cancer 1982; 29(5): 539-45 Tavassoli et al, Am J Surg Pathol. 1990; 14(2): 128-33 Thor et al, Sem Oncol 1986; 13(4): 393-401
107	Antigen	Tn-associated antigen	Konska et al, Int J Oncol 1998; 12(2): 361-7
108		N-acetyl-lactosamine	Konska et al, Int J Oncol 1998; 12(2): 361-7
109	Lectin	Lectin	Konska et al, Int J Oncol 1998; 12(2): 361-7
110	Receptor	Lectin receptor	Konska et al, Int J Oncol 1998; 12(2): 361-7
111	Antibody Antigen	Mab detecting 83D4 antigen	Pancino et al, Hybridoma 1990; 9(4):389-395 Beuzelin-Yuraut et al, J Clin Pathol 1995; 48: 433-437
112	Antigen Proteolipid	SP-2 (90 kD antigen)	Iacobelli et al, Cancer Res. 1986: 46: 3005-3010
113	Antibody Antigen	Mab 323/A3 (detecting Mr 43kD glycoprotein)	Edwards et al, Cancer Res 1986; 46(3): 1306-17 Courtney et al, Cancer Lett 1991; 57(2): 115-9
114	Antigen	Ca-1	Courtney et al, Br J Cancer Suppl 1990; 10: 92-5
115	Antibody Antigen	T-antigen MBE6 antibody	Teramoto et al, Cancer 1982; 50: 241-249
116	Receptor	c-met tyrosine kinase receptor	Nakopoulou et al, Histopathology 2000; 36(4):313-25
117	Growth Factor Ligand	Hepatocyte growth factor (HGF)	Nakopoulou et al, Histopathology 2000; 36(4):313-25 Qiao et al, Cell Growth Diff 2000;11(2):123-33
118	Ligand Nucleic Acid	Angiopoietin-1	Huang et al, Blood 2000;95(6):1993-9
119	Nucleic acid Protein	Nm23	Guo et al, Chung Hua I Hsueh I Chuan Hsueh Tsa Chih 2000; 17(2): 91-93 Midulla et al, Anticancer Res 1999; 19(5B):4033-7
120	Protein	Ki67	Midulla et al, Anticancer Res 1999; 19(5B):4033-7
121	Protein	P21	Reed et al, Virchows Arch 1999; 435(2); 116-24

122	Protein	P27	Reed et al. Virchows Arch 1999; 435(2); 116-24
123	Protein Antibody	TKH1 TKH2	Kjeldsen et al, Cancer Res 1988; 48(8):2214-20
124	Antigen Disaccharide	Sialosyl-Tn	Kjeldsen et al, Cancer Res 1988; 48(8):2214-20
125	Enzyme	Lactate Dehydrogenase (LDH)	Kher et al, Indian J Pathol Microbiol 1997; 40(3): 321-6
126	Enzyme	Myosin light chain kinase	Nguyen et al, J Cell Biol 1999; 146(1): 149-64
127	Tumor suppressor	beta2 microglobulin	Carmon et al, Int J Cancer 2000; 85(3):391-7
128	Protein Peptide	MUC1	Carmon et al, Int J Cancer 2000; 85(3):391-7

EXAMPLES

1. Retrieval of Ductal Fluid and Analysis of Markers in the Fluid

A patient is prepared for a ductal lavage, using a ductal access tool and a duct on each breast is lavaged and the ductal fluid collected separately from each accessed duct. The fluid in each duct that is accessed is analyzed for nuclear matrix proteins, maspin, claudin 7, telomerase, basic FGF, fibrinogen degradation peptide, and CSF-1 receptor using standard techniques.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims